Appl. No. 10/791,592
Amdt. dated March 1, 2005
Reply to Office Action of February 4, 2005

Amendments to the Specification:

Please replace paragraph [0020] with the following amended paragraph:

-- FIG. 1**A-1D** illustrates the human cDNA and amino acid sequences (SEQ ID NO: 1 and SEQ ID NO: 2, respectively) of the isolated MCP-1 receptor clone, MCP-1 RA. --

Please replace paragraph [0021] with the following amended paragraph:

-- FIG. 2**A-2C** illustrates the human cDNA and amino acid sequences (SEQ ID NO: 3 and SEQ ID NO: 4, respectively) of the isolated MCP-1 receptor clone, MCP-1RB. --

Please replace paragraph [0022] with the following amended paragraph:

-- FIG. 3**A-3B** illustrates the results of Northern blot analysis of hematopoietic cell lines that were probed for MCP-1RA and MCP-1RB mRNA. --

Please replace paragraph [0023] with the following amended paragraph:

-- FIG. 4**A-4B** illustrates the predicted amino acid sequence of the MCP-1 receptor A (MCP-1RA) (SEQ ID NO: 2), aligned with the MIP-1α/RANTES receptor sequence (SEQ ID NO: 5), the orphan receptor sequence HUMSTSR (SEQ ID NO:6) and the two IL-8 receptor seqeunces (SEQ ID NOS: 7 and 8). Identical residues are boxed. The seven putative transmembrane domains are indicated by the horizontal bars. Gaps inserted to optimize the alignments are indicated by dashes. Amino acid numbers for each sequence are located to the right of the sequences. --

Please replace paragraph [0026] with the following amended paragraph:

-- FIG. 7**A-7B** graphically depicts the binding of ^{125}I-MCP-1 to the recombinant MCP-1RB receptor, as described in detail in Example 5. --

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Please replace paragraph [0027] with the following amended paragraph:

-- FIG. 8A-8B graphically depicts the results of the MCP-1RB receptor-mediated calcium mobilization experiments also described in detail in Example 5. FIG. 8A depicts intracellular calcium flux as a function of MCP-1 concentration (nM). Calcium transients peaked at 4-8 sec. after addition of MCP-1 and returned to baseline within 90 sec. of activation. FIG. 8B depicts the MCP-1 stimulated calcium mobilization (EC_{50} = 3.4 nM) and the lack of stimulated calcium mobilization by other cytokines. 8C illustrates that MCP-1 desensitized the cells to a second addition of MCP-1. --

Please replace paragraph [0030] with the following amended paragraph:

-- To obtain a full-length version of this clone, an MM6 cDNA library was constructed and probed with the PCR product. An isolated clone of 2.1kb was obtained and called MCP-1RA. FIG. 1 ~~Illlustrates~~ illustrates the cDNA sequence (SEQ ID NO: 1) and the predicted amino acid sequence (SEQ ID NO: 2) of the clone. The nucleotide sequence (SEQ ID NO: 1) comprises 2232 base pairs, including a 5' noncoding sequence of 39 base pairs and a 3' noncoding sequence of 1071 base pairs. The MCP-1RA sequence is characterized by a single long open reading frame encoding a 374 amino acid following the initiation methionine at position 23. --

Please replace paragraph [0066] with the following amended paragraph:

-- Sequences encoding metabolic pathway enzymes provide particularly useful promoter sequences. Examples include promoter sequences derived from sugar metabolizing enzymes, such as galactose, lactose (lac) (see Chang, Nature 198:1056 (1977), and maltose. Additional examples include promoter sequences derived from biosynthetic enzymes such as tryptophan (tip) (see Goeddel, NUC. ACIDS RES. 8:4057 (1981), Yelverton, Nuc. Acids Res. 9:731 (1981), U.S. Patent No. 4,738,921 and EP Patent Pub. Nos. 36 776 and 121 775). The [[▪]] beta-lactomase (bla) promoter system (see Weissmann, Interferon 3 (ed. I. Gresser), the bacteriophage lambda PL promoter system (see Shimatake, Nature 292:128 (128) and the T5 promoter system (U.S. Patent No. 4,689,406) also provides useful promoter sequences. --

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Please replace paragraph [0083] with the following amended paragraph:

-- A method is provided for identifying ligands of the MCP-1 receptor, such as antagonists. The method comprises transfecting a mammalian cell line with an expression vector comprising nucleic acid sequences encoding the N-terminal domain of MCP-1 receptor (see SEQ ID NOS: 1 and 3). The N-terminal domain of the MCP-1 receptor may be expressed alone or in combination with other domains of the MCP-1 receptor. The other domains may be extracellular, intracellular or transmembrane domains. Moreover, a chimaeric protein may be expressed, where the other domains are the corresponding domains from related proteins, such as those in ~~Fig. 4,~~ **FIG. 4** (SEQ ID NOS: 5, 6, 7 and 8). The N-terminal domain may also be expressed as a portion of the native MCP-1 receptor. Expression of extracellular domains is preferred where soluble protein for solid phase assays is required. --

Please replace paragraph [0113] with the following amended paragraph:

-- Equilibrium binding data were analyzed according to the method of Scatchard using the program "LIGAND" (Biosoft, Ferguson, MO) on a Macintosh computer. See Munson, Anal. Biochem. 107: 220-39 (1980). The closely related C-C chemokines MIP-1α, MIP-1β, and RANTES, as well as the C-X-C chemokine IL-8 did not compete for binding. Nor was specific binding detected in transfectants that expressed little or no MCP-1RB on Northern blots. Analysis of equilibrium binding data shown in ~~Fig. 7~~ **FIG. 7** indicates a dissociation constant (K_d) of 260 pM (Fig 7B). This K_d is in good agreement with that reported for the binding of MCP-1 to monocytes (Yoshimura, J. Immunol. 145:292-97 (1990); Zhang, J. Biol. Chem. 269:15918-24 (1994)) and THP-1 cells (Van Riper, J. Exp. Med. 177:851-56 (1933)). These data indicate that ^{125}I-MCP-1 bound specifically and with high affinity to the MCP-1RB receptor expressed in 293 cells. --

Please replace paragraph [0118] with the following amended paragraph:

-- MCP-1 stimulated robust calcium mobilization in the stably transfected MCP-1RB/293 cells in a specific and dose-dependent manner. Small but reproducible signals were seen with as little as

100 pM MCP-1, and the average EC_{50} from four full dose-response curves to MCP-1 was 3.4 nM (2.7-4.4 nM; ~~Fig. 8, A and B~~ **FIG. 8A and 8B**). --

Please replace paragraph [0119] with the following amended paragraph:

-- The MCP-1RB receptor was selectively activated by MCP-1. RANTES, MIP-1α, MIP-1β, Gro-α, and IL-8 failed to stimulate significant calcium signals in these same cells, even when present at high concentrations (~~Fig. 8B~~ **FIG. 8B**). Furthermore, these chemokines also failed to block stimulation of the cells by MCP-1, indicating that they are unlikely to act as endogenous antagonists of the MCP-1RB receptor. The MCP-1-dependent intracellular calcium fluxes were characterized by short lag times, followed by a rapid rise in $[Ca^{2+}]_i$ that returned to near basal levels within 80-90 sec of the addition of MCP-1 (~~Fig 8A~~ **FIG. 8A**). The cells demonstrated homologous desensitization in that they were refractory to activation by a second challenge with MCP-1 (~~Fig. 8C~~ **FIG. 8C**). --